US Army Corps
of Engineers
Construction Engineering
Research Laboratories

USACERL Technical Report 95/44
September 1995

Biotechnology To Separate and Treat Metals in Sludge and Wastewater

A Literature Review

by
Byung J. Kim, Daniel K. Cha, and June S. Song

Army industrial sludge may be classified as a hazardous waste when it contains oil and grease, metals, and energetic compounds. It is difficult to treat this hazardous waste according to regulatory requirements at a reasonable cost using conventional sludge treatment methods. Biologic separation/ treatment of metals from industrial sludge has been identified as a possible alternative to conventional technologies for treating industrial sludge.

Biologic treatment of sludge uses naturally occurring biochemical reactions in which pollutants can be used as resources. The process offers a low-cost, highly efficient alternative to existing sludge treatment methods. This report summarizes a literature review that examined the development and status of biotechnology to separate and treat metals in sludge and wastewater.

The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products. The findings of this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DESTROY THIS REPORT WHEN IT IS NO LONGER NEEDED

DO NOT RETURN IT TO THE ORIGINATOR

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1995	Final

4. TITLE AND SUBTITLE
Biotechnology To Separate and Treat Metals in Sludge and Wastewater: A Literature Review

5. FUNDING NUMBERS
4A162720
D048
UZ3

6. AUTHOR(S)
Byung Kim, Daniel K. Cha, and June S. Song

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Construction Engineering Research Laboratories (USACERL)
P.O. Box 9005
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
TR 95/44

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Headquarters, U.S. Army Corps of Engineers (HQUSACE)
ATTN: ENAEC-TS
20 Massachussetts Ave., NW
Washington, DC 20314-1000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Copies are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Army industrial sludge may be classified as a hazardous waste when it contains oil and grease, metals, and energetic compounds. It is difficult to treat this hazardous waste according to regulatory requirements at a reasonable cost using conventional sludge treatment methods. Biologic separation/treatment of metals from industrial sludge has been identified as a possible alternative to conventional technologies for treating industrial sludge.

Biologic treatment of sludge uses naturally occurring biochemical reactions in which pollutants can be used as resources. The process offers a low-cost, highly efficient alternative to existing sludge treatment methods. This report summarizes a literature review that examined the development and status of biotechnology to separate and treat metals in sludge and wastewater.

14. SUBJECT TERMS
biotechnology
heavy metals
industrial wastewater treatment
hazardous wastes

15. NUMBER OF PAGES
46

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	SAR

Foreword

This study was conducted for Headquarters, U.S. Army Corps of Engineers (HQ-USACE) under Project 4A162720D048, "Industrial Operations Pollution Control Technology"; Work Unit UZ3, "Sludge Treatment at AMC Installations." The technical monitor was Theodore Ruff, ENAEC-TS.

The work was performed by the Industrial Operations Division (UL-I) of the Utilities and Industrial Operations Laboratory (UL), U.S. Army Construction Engineering Research Laboratories (USACERL). The USACERL principal investigator was Dr. Byung Kim. Daniel Cha and June Song are associated with the Prizker Department of Environmental Engineering, Illinois Institute of Technology, Chicago. Ralph E. Moshage is Chief, CECER-UL-I, John T. Bandy is Operating Chief, and Gary W. Schanche is Chief, CECER-UL. The USACERL technical editor was Agnes E. Dillon, Technical Resources Center.

COL James T. Scott is Commander and Acting Director of USACERL. Michael J. O'Connor is Technical Director.

Contents

1 Introduction

Background

Army industrial sludge that contains oil and grease, metals, and energetic compounds, may be classified as hazardous waste. The Army has difficulty disposing of such hazardous waste according to regulatory requirements at a reasonable cost using conventional sludge treatment methods. Moreover, conventional techniques—such as chemical precipitation, electrolysis, ion exchange for polluted water and acid extraction, heat treatment, and liquid ion exchange for polluted solids—are likely to become increasingly expensive and inefficient when anticipated stricter regulations are introduced. A recent study by the U.S. Army Construction Engineering Research Laboratories (USACERL) assessed the current status of Army industrial sludge management, identified technologies to improve sludge management, and recommended further research (Kim et al., September 1995). In the study, biologic separation/treatment of metals from industrial sludge was identified as a possible alternative to conventional sludge treatment technologies.

Biologic remediation of environmental pollution problems has received considerable attention in recent years. A significant amount of research has been conducted on the topic, but this research has focused primarily on organic pollutants as opposed to inorganic pollutants. The scarce application of biologic approaches for the treatment of inorganic pollutants, e.g., heavy metals, is chiefly due to their nonbiodegradability. Nevertheless, researchers are still attempting to develop effective treatment technologies for heavy metal removal that use microorganisms and microbial products. Biologic treatment approaches use the naturally occurring biochemical reactions in which pollutants can be used as resources; this offers a highly efficient, relatively low-cost alternative to existing methods for heavy metals detoxification and recovery. This report summarizes a literature review that examined the development and status of biotechnology to separate and treat metals in sludge and wastewater

Objectives

This literature review was conducted as part of a larger study on sludge management (Kim et al., September 1995). The objectives of this part of the study were to provide

additional information on current sludge management practices and to summarize the current status of biotechnology to separate and treat metals in sludge and wastewater.

Approach

A literature review was conducted, focused specifically on the development and status of microbiological applications in the area of metal removal from polluted water and solids.

Mode of Technology Transfer

It is anticipated that the results of this study will form the basis for future 6.2 research and development efforts. This information also will be used by the Army industrial community (depots, arsenals, and ammunition plants) as the theoretical basis for future improvement of metal-containing sludge or wastewater treatment processes.

Reference

Kim, B.J., G. Hunter, E. Kobylinski, and P. Martin, *Army Industrial Sludge Management Technologies: Evaluation and Recommendations for Improvement,* Technical Report 95/42 (U.S. Army Construction Engineering Research Laboratories [USACERL], September 1995).

2 Microbial Leaching of Heavy Metals From Contaminated Solids

For the last 20 years, various chemical methods have been studied for heavy metal removal from sludges: chlorination (Olver, Kreye, and King 1975), ion exchange (Cornwell and Westernoff 1980), utilization of chelating agents such as EDTA (Bloomfield and Pruden 1975), nitrilotriacetic acid (NTA), aerobic autoheated thermophilic digestion (AATD) coupled with hydrochloric acid (Hayes, Jewell, and Kabrick 1980), and oxidative acid hydrolysis (Kiff and Brown 1981). In spite of good metal extraction achieved by some chemical treatment methods, factors such as cost, operational difficulties, and a large volume of chemical usage have made practical application of these methods unattractive (Tyagi, Couillard, and Tran 1988). For example, the acid treatment method not only requires a large amount of acid (0.5 to 0.6 g of H_2SO_4/dry weight of sludge) during the treatment, but it also requires lime at the end of the treatment. In some instances, chemical methods alone cannot give acceptable removal efficiencies. An alternative to a chemical method is the use of microorganisms to remove heavy metals from contaminated solids; this process commonly is referred to as "microbial leaching."

Metabolic Mechanism of Bioleaching

Microbial leaching is a biochemical oxidation process that utilizes microorganisms to solubilize insoluble inorganic substrates. Metals can be dissolved from insoluble sulfide minerals directly by the metabolism of microorganisms or indirectly by the products of microbial metabolism.

Indirect Mechanism

Two important reactions mediated by one of the most studied iron-oxidizing bacteria, *Thiobacillus ferrooxidans*, are:

$$FeS_2 \text{ (pyrite)} + 3.5\,O_2 + H_2O \rightarrow FeSO_4 + H_2SO_4 \quad (1)$$

$$2\,FeSO_4 + 1.5\,O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O \quad (2)$$

Ferric sulfate [$Fe_2(SO_4)_3$] is a strong oxidant capable of dissolving a wide variety of metal sulfide minerals (Hutchins et al. 1986). Ferric sulfate leaching is termed the "indirect" leaching mechanism because it proceeds even in the absence of oxygen or viable bacteria; it is responsible for solubilizing economically important metals from mineral ore. For example, copper can be leached from copper sulfide minerals by the following reactions (Hutchins et al. 1986):

$$CuFeS\ (chalcopyrite) + 2\,Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5\,FeSO_4 + 2\,S^\circ \quad (3)$$

$$Cu_2S\ (chalcocite) + 2\,Fe_2(SO_4)_3 \rightarrow 2\,CuSO_4 + 4\,FeSO_4 + S^\circ \quad (4)$$

$$CuS\ (covellite) + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2\,FeSO_4 + S^\circ \quad (5)$$

$$Cu_5FeS_4\ (bornite) + 6\,Fe_2(SO_4)_3 \rightarrow 5\,CuSO_4 + 13\,FeSO_4 + 4\,S^\circ \quad (6)$$

Ultimately, the indirect leaching mechanism depends on biological regeneration of ferric sulfate (reaction 2). Elemental sulfur (S°) generated by reactions 3 to 6 can be converted to sulfuric acid by *T. ferrooxidans*:

$$2\,S^\circ + 3\,O_2 + 2\,H_2O \rightarrow 2\,H_2SO_4 \quad (7)$$

This production of sulfuric acid further maintains the pH at levels favorable to the bacteria solubilizing a variety of metal oxides.

Direct Mechanism

Iron-oxidizing bacteria also leach metal sulfides "directly" without involvement of microbially produced ferric sulfate. Although the direct leaching mechanism is inferred from observations of microbial attachment to metal sulfides, the best evidence comes from studies using synthetically prepared metal sulfide minerals free of iron (Torma 1971). Incubation of cells with these synthetic minerals results in oxygen

consumption and metal solubilization. The process is described by the simplified reaction:

$$MS + 2O_2 \rightarrow MSO_4 \quad (8)$$

where M represents a divalent metal. Torma (1971) reported that the addition of 10^{-2} to 10^{-4} M ferric iron to synthetic metal sulfide minerals doubled metal extraction rates. Because iron nearly always is available in natural leaching environments, both direct and indirect leaching mechanisms probably occur simultaneously.

Both direct and indirect mechanisms contribute to the bioleaching of metal sulfides. Relative contributions of each degradative mechanism is determined by a number of factors that include the physical and chemical characteristics of individual minerals, the particular microbial agents, and the nature of the physicochemical environment. Murr (1980) has extensively reviewed the leaching of copper ores and concluded that the pyrite commonly associated with copper sulfide minerals is degraded predominantly by the indirect mechanism. Similarly, the studies by Smith and Shumate (1970) suggested that the pyrite oxidation responsible for acid coal mine drainage is generally indirect.

The classification of microbial interventions in bioleaching processes into "direct" and "indirect" types is oversimplistic and not particularly illuminating. It does not accommodate the complexity and diversity of the microbial degradation modes than can occur in bioleaching of metal sulfide. The terms "direct" and "indirect" do not clarify the intimate mechanisms involved, except to imply that the first type must occur in close proximity to the metal sulfide surface; the second type may operate elsewhere. Regardless of either mechanism, the key factor that influences the bioleaching is the microbial activity.

A number of modes of biological intervention have been demonstrated by various researchers:

1. Facilitation of the mass transfer of reactants by mere effective wetting of the metal sulfide surface by surface active agents excreted by the microorganisms. A number of such agents have been related to the activity of *Thiobacillus* species.

2. Removal of passivation layers on the metal sulfide surface arising from chemical or electrochemical reactions.

3. Shifting of dissociation equilibria on metal sulfide surfaces. Torma and Sakaguchi (1978) suggested that the first step in metal sulfide oxidation is the dissociation of the substrate as follows:

$$MS \rightarrow M^{+2} + S^{-2} \quad (9)$$

(This was based on a direct correlation between the rates of bacterially mediated oxidation of a number of synthetic metal sulfides and their solubility products.)

The released sulfide moiety is being immediately captured by the enzymatic system of the bacteria, shifting the dissociation reaction toward completion. Tributsch and Bennett (1981) further examined this concept and concluded thatthe most critical rate-determining parameters (in the absence of any electron acceptors, e.g., Fe^{+3}) was the solubility product of the sulfide.

4. Production of chelating agents by the microbial agents. Possible metal-chelating capabilities of the extracellular materials excreted by *T. ferrooxidans* have not been extensively investigated. Biogenic acids such as citric, glutamic, and 2-ketoglutamic are known to have strong chelating or binding capacity for metals and other cations (Silverman 1979).

5. Modification of physicochemical parameters. Principal physicochemical factors that influence the rate of the chemical degradation reactions and the level of microbial activities are temperature, pH, and oxidation-reduction potential (Eh), as well as other factors such as particle size and surface area of the metal sulfide substrate and the concentrations of nutrients and reactants.

Microbiology of Iron-Oxidizing Bacteria

For many years the only microorganism thought to be responsible for the leaching of metals was *T. ferrooxidans*. In recent years other iron-oxidizing microorganisms, such as *Leptospirllum ferrooxidans* and unique thermophilic bacteria, have been discovered and characterized. Among these bacteria, *T. ferrooxidans* has been the most widely studied and is currently the major leaching microorganism for the mining industry (Torma 1971).

T. ferrooxidans is a Gram-negative, acidophilic, mesophilic chemoautroph. Cells are short, straight rods approximately 1.0 μm long and 0.5 μm in diameter. Some strains

have flagella and/or pili (Dispirito et al. 1982). *T. ferrooxidans* is most active in a pH range of 1.5 to 3.5 with an optimum pH of 2.3. It prefers a temperature range of 30 to 35 °C and has an activity to oxidize both Fe (II) and reduced sulfur compounds. Many strains of *T. ferrooxidans* have been isolated that have different capacities to oxidize certain substrates, different DNA base composition, and different resistances to metal ions and acidity (Hughes and Poole 1989).

Physiology of Metal-Leaching Bacteria

T. ferrooxidans, as is true of other thiobacilli, derives the balance of its biosynthetic carbon requirements from carbon dioxide via the Calvin-Benson cycle and carboxylation of phosphenol pyruvate. Oxidation of either ferrous iron or reduced sulfur compounds provides the energy required for carbon dioxide fixation and other functions in intermediary metabolism of *T. ferrooxidans* (Hutchins et al. 1986).

The ferrous iron oxidation system of *T. ferrooxidans* is associated with the cellular membrane/envelope. Cobley and Haddock (1975), using purified enzyme and soluble iron substrates, suggested that the key iron-oxidizing enzyme appears to be Fe^{+2}-cytochrome c oxidoredutase; cytochrome a and coenzyme Q also appear to be associated with the process. Cox and Boxer (1978) identified a copper-containing protein, rusticyanin, that serves as the initial electron acceptor for oxidation of ferrous iron. The relationship between rusticyanin and Fe^{+2}-cytochrome c reductase needs to be further investigated (Hutchins et al. 1986).

The mechanism of sulfur oxidation by *T. ferrooxidans* is similar to that of other thiobacilli. Elemental sulfur, tetrathionate, thiosulfate, and the sulfide moiety of various noniron minerals support the growth of the organism, implying sulfite (SO_3^{-2}) is the key intermediate molecule (Vestal and Lundgren 1971). Energy is produced from sulfate oxidation mediated by the interaction of the enzymes sulfite oxidase, ADP sulfurylase, APS reductase, and adenylate kinase.

Sugio et al. (1985) proposed an alternative sulfur-oxidation mechanism in which elemental sulfur is oxidized to sulfite then to sulfate via the ferric iron reduction system of *T. ferrooxidans*. Researchers indicated that this alternatives mechanism may be important under conditions of oxygen-limited growth conditions.

Metal Resistance of the Microbes

The iron- and sulfur-oxidizing mechanisms of *T. ferrooxidans* generally are insensitive to high concentrations of metal cations and oxyanions (Brierley 1978; Lundgren and Silver 1980). Ferrous iron oxidation is possible in the presence of zinc (0.15 M) , nickel (0.17 M), copper (0.16 M), cobalt (0.17 M), manganese (0.18 M), and aluminum (0.37 M). Tuovinen et al. (1971) found that uranium and the anions, arsenic, selenium, and tellurium are inhibitory at a concentration of 0.8 to 3 mM. Molybdate inhibits iron oxidation above 0.05 mM concentration, and mercury and silver are toxic at concentrations as low as 0.5 μM. Kelly and Jones (1978) discussed the inhibitory effects of both ferrous and ferric iron concentrations on iron oxidation and carbon dioxide assimilation. Other toxic metals that have been investigated include gold, chromium, thallium, and rubidium (Razzell and Trussel 1963).

Resistance of bacteria to metal cations and oxyanions is usually plasmid-mediated. Plasmid-mediated resistance to mercury, tellurium, arsenic, silver, nickel, cobalt, zinc, cadmium, copper, and lead has been reported for heterotrophic bacteria whose pH optima are near neutral (Hutchins et al. 1986). Little is known regarding the nature of metal resistance in acidophilic iron-oxidizing bacteria.

Hutchins et al. (1986) reported that threshold levels of tolerance for metal ions depend on the specific strain and physiological state of the organism, its previous history of exposure to the toxicant, environmental conditions during exposure, and the empirical method by which tolerance and toxicity are determined. Tolerance levels frequently can be increased by subculturing the organisms in progressively higher concentrations of the toxic ion. Resistance conferred by adaptation to one metal either can enhance or decrease the organism's tolerance to other metals. Susceptibility of organisms to the toxicity of some metals is higher during thiosulfate oxidation than during ferrous iron and sulfur oxidation. This difference was attributed to physiological differences in membrane structure and function as a result of growth on the different substrates (Hutchins et al. 1986).

The impact of metals toxicity on *T. ferrooxidans* on bacterial leaching is difficult to assess in natural leaching environments because metal cations gradually accumulate in solution. Although metal concentrations can become inhibitory, toxic effects may be lessened through adaptation of the leaching bacteria, precipitation of toxic metals, formation of soluble metal complexes, sorption of metals to particles, and other governing parameters and processes (Brierley 1978). The possible synergistic and antagonistic effects of solubilized metals on the activity of *T. ferrooxidans* need to be further investigated.

Parameters Affecting Bacterial Leaching

Temperature

Metal leaching by microorganisms is confined to a limited range of temperature. Increasing the temperature results in the usual augmentation of the chemical reaction rate as well as in faster microbial metabolism. Optimum leaching of metal sulfide and oxidation of ferrous iron by iron-oxidizing bacteria have been determined to occur between 25 and 45 °C (Lundgren and Silver 1980). Although the lower temperature limits of the metabolic activity of the iron-oxidizing bacteria have not yet been established, the lower limit of this activity is generally accepted to be the freezing point of water.

pH

T. ferrooxidans is active in the pH range of 1.5 to 5. Optimum pH for bacterial leaching of metal sulfides varies with the nature of the substrate and is within the range of 1.0 to 2.5. Karavaiko (1985) suggested that high pH values may affect the bacterial cell wall and hence influence the direct bacterial leaching process.

Thermophilic *Sulfolobus* bacteria have been shown to be active between pH values of 1 to 6, with the optimum pH between 2 and 3 (Brock et al. 1972). A number of thermophilic thiobacilli also have been isolated with various pH optima between 2 and 9 (LeRoux, Wakerley, and Hunt 1977).

Oxidation-Reduction Potential

Indirect oxidation that results in solubilization of minerals by ferric iron is highly dependent on oxidation-reduction potential (ORP). ORP values between +190 and +550 mV have been measured during this oxidation (Lundgren and Silver 1980). Similarly, ORP values of between +220 and +515 mV and between +340 and +540 mV was observed during the oxidation of metal sulfides and the extraction of uranium, respectively, by the iron-oxidizing thiobacilli.

ORP values of metal-laden solids can be raised either by chemical oxidation or biological oxidation. Chemical oxidation can be achieved with aeration or by the addition of an oxidizing agent (Tyagi, Couillard, and Tran 1988).

Nutrients

Bacterial growth requires nitrogen (NH_4^+), potassium, calcium, phosphorus (PO_4^{-3}), iron ($FeSO_4$), sulfate (SO_4^{-2}), magnesium, and trace elements. Growth media for the *Thiobacilli* and the thermophiles contain these substances in various quantities. Laboratory studies usually use synthetic medium containing the essential nutrients; however, they obviously are too expensive for use in full-scale operations. Accurate identification and quantification of required nutrients are desirable for full-scale application of the bioleaching process. The organism's requirement for sulfur is fulfilled by sulfate, which is a product or byproduct of the oxidation of metal sulfide. Potassium, calcium, and magnesium usually are present in leaching media in excess of the minimum requirements needed for growth. Phosphate and ammonia generally are the only constituents that are not ubiquitous (Lundgren and Silver 1980).

Air

Aeration provides both oxygen and carbon dioxide for the activity of bioleaching bacteria. Iron-oxidizing thiobacilli are strictly aerobic; limitations in availability of oxygen affect the rates of ferrous iron oxidation and metal leaching. Wong and Henry (1984) reported that the ferrous iron oxidation and metal extraction from metal sulfides were stimulated when the aeration levels were increased. Conversely, when CO_2 was reduced, the rate of ferrous oxidation decreased.

Substrate Surface Area and Solid Concentration

Specific surface area is one of the important factors that influences the bacterial leaching process. The rate of metal extraction from ores as well as industrial sludge increases with a decrease in the particle size. Eligwe (1988) observed that the rate of dissolution of iron from pure pyrite was exponentially dependent on the diameter of the pyrite.

Microbial leaching also is dependent on solids concentration. Higher solids concentrations result in more available substrate and tend to increase the absolute quantity of metal values solubilized. However, the rate of bioleaching may decrease when the solid liquid ratio is high because, with the high solids, the mass transfer of nutrients, especially oxygen and carbon dioxide, to the organisms is limited.

Bioleaching of Metals From Anaerobically Digested Biosolids

Application of wastewater sludge on agricultural land is an attractive sludge management option because it combines beneficial reuse and disposal. However, it is important to reduce the metal contents in the sludge to minimize the health hazard associated with metal uptake by plants and its subsequent accumulation in the food chain.

Wong and Henry (1983a) demonstrated that bioleaching is a reliable technology for removal of heavy metals from digested sludge. In batch experiments, metal solubilization of 86, 65, 78, and 87 percent were obtained for Cd, Cu, Ni, and Zn, respectively, in 10 days of bioleaching at 20 to 25 °C. Acid requirement was about 0.15 g H_2SO_4/g dry weight of sludge. To achieve similar levels of removal for Cd, Ni, and Zn using acid treatment alone required an acid dosage of more than 0.7 g of H_2SO_4/g dry weight of sludge.

Wong and Henry (1983b) found that aeration of 50 cm^3 of air per liter of sludge per minute was adequate for bacterial leaching of metals from digested sludge. Metal removal efficiency was found to increase by about 30 percent when the temperature was increased from 10 to 25 °C. Blais, Tyagi, and Auclair (1993b) showed that the growth of leaching bacteria was temperature dependent for a temperature interval of 7 to 28 °C.

Organometal complexation also can affect metal solubilization from sludge. Wong and Henry (1984) reported that the efficiency of metal solubilization followed the sequence: Cd = Zn > Ni > Cu > Pb. The stability of organometal complexation followed the reverse order. The fact that Pb forms very stable organometal complexes explained the low removal of Pb from the sludge during bacterial leaching.

Blais, Tyagi, and Auclair (1993b) studied the metal removal from municipal biosolids by acid treatment and two microbial leaching processes in laboratory reactors. Microbial leaching processes with elemental sulfur and ferrous sulfate as substrate reduced the quantity of acid required for metal extraction by 100 and 83 percent, respectively. Bioleaching processes using sulfur-oxidizing bacteria with sulfur as substrate (bioreaction time of 5 days) was found to be more efficient than microbial leaching processes with iron-oxidizing bacteria (bioreaction time of 10 days) for solubilization of cadmium, copper, manganese, and zinc.

Blais, Tyagi, and Auclair (1993b) further characterized the naturally occurring microorganisms responsible for the metal leaching activity in 21 different wastewater sludges. Isolation and identification of the bacterial strains revealed that acid production is caused by the presence of thiobacilli. The initial acidification of the

sludge in the process of bioleaching is brought about by the growth of indigenous, less-acidophilic thiobacilli followed by the growth of acidophilic thiobacilli; this results in a pH reduction to about 2.0. *T. thiooxidans* was the only species in acidifying microfloras that belongs to acidophilic thiobacilli, and *T. thioparus* was the dominant species for the initial sludge acidification.

Blais, Tyagi, and Auclair (1993a) determined the growth kinetics of the two groups of thiobacilli in five different wastewater sludges. After 5 days of incubation in shake flasks, the pH of the sludges decreased to about 2.0; this pH decrease solubilized various toxic metals (83 to 90 percent Cd, 19 to 41 percent Cr, 69 to 92 percent Cu, 88 to 99 percent Mn, and 77 to 88 percent Ni). The maximum specific growth rate (μ_{max}) for the less-acidophilic thiobacilli varied between 0.079 and 0.104 h^{-1}; that for the acidophilic thiobacilli varied between 0.067 and 0.079 h^{-1}. Batch growth and sulfate production studies showed that the sludge type and sludge solids content did not significantly affect the development of thiobacilli.

Couillard and Mercier (1991) compared the behavior of metal solubilization in a continuously stirred tank reactor (CSTR) and an airlift reactor. Similar leaching efficiencies were observed for both reactors operating at hydraulic residence time of 0.75 days. At steady state, 91 percent Cu, 94 percent Zn, 93 percent Mn, 67 percent Ni, and 67 percent Cd were solubilized in the CSTR; and 89 percent Cu, 91 percent Zn, and 82 percent Cd were solubilized in the airlift reactor.

Sreekrishnan et al. (1993) reported that sludge solids concentration indirectly influences the acid production by regulating the nature of the pH drop in the system. The inter-relationship between sludge solids concentrations, sulfate concentration, and medium pH has a significant impact on the performance of the bioleaching process. Sreekrishnan et al. (1993) introduced a new system parameter called buffering capacity index (BCI) to describe the effect of this inter-relating variables. BCI was found to have a linear relationship with sludge solids concentration for a given type of sludges.

Tyagi et al. (1993) developed a conceptual model for bioleaching of metals from wastewater sludge by selecting a quantitative relationship among the various process parameters. Sludge pH was identified as the parameter that controls bacterial growth and thus the overall process. Bacterial growth, sulfate concentration, and pH profiles simulated from the model closely matched experimental observations. The major shortcoming of the model is that it only predicts the sulfate concentration; it does not predict the actual dissolved metal concentration. The researchers stated that the metal solubilization process could not be incorporated directly into the model because of various unquantified factors influencing the leaching process. To partially offset

this shortcoming, they developed empirical solubility charts to predict the metal solubilization.

Konishi, Kubo, and Asai (1991) reported the leaching kinetics of ZnS by *T. ferrooxidans* in a batch reactor. The adsorption rate of the bacteria was fairly rapid in comparison with the bioleaching rate, indicating that the bacterial adsorption is at equilibrium during the leaching process. In the absence of bacteria, the rate of metal leaching varied with the concentration of ferric iron and the first-order reaction rate constant of $0.632/d/m^2$.

Cited References

Blais, J. F., R.D. Tyagi, and J.C. Auclair, "Bioleaching of metals from sewage sludge: microorganisms and growth kinetics," *Water Research,* vol 27 (1993a), p 101.

Blais, J.F., R.D. Tyagi, and J.C. Auclair, "Bioleaching of metals from sewage sludge: effects of temperature," *Water Research,* vol 27 (1993b), p 111.

Bloomfield, C., and G. Pruden, "The effects of aerobic and anaerobic incubation on extractibility of heavy metals in digested sludge," *Environmental Pollution*, vol 8 (1975), p 217.

Brierley, C.L., "Bacterial leaching," *CRC Critical Reviews in Microbiology*, vol 6 (1978), p 207.

Brock, T.D., K.M. Brock, R.T. Belly, and L.R. Weiss, "*Sulfolobus*: a new genus of sulphur-oxidizing living at low pH and high temperature," *Archiv fuer Mikrobiologie,* vol 84 (1972), p 54.

Cobley, J.C., and B.A. Haddock, "The respiratory chain of *Thiobacillus ferrooxidnas*: the reduction of cytochromes by Fe2+ and the preliminary characterization of rusticyanin a novel "blue" copper protein," *Federation of European Biochemical Societies Letters,* vol 60 (1975), p 29.

Cornwell, D.A., and G.P. Westernoff, "Extract heavy metals via liquid-ion exchange," *Water and Waste Engineering* (February 1980), p 36.

Couillard, D., and G. Mercier, "Optimum residence time (in CSTR and airlift reactor) for bacterial leaching of metals from anaerobic sewage sludge," *Water Research,* vol 25 (1991), p 211.

Cox, J.C., and D.H. Boxer, "The purification and some properties of rusticyanin, a blue copper protein involved in iron (II) oxidation from *Thiobacillus ferrooxidans*," *Biochemical Journal*, vol 174 (1978), p 497.

DiSpirito, A.A., M. Silver, L. Voss, and O.H. Tuovinen, "Flagella and pili of iron-oxidizing thiobacilli isolated from a uranium mine in Northern Ontario, Canada," *Applied and Environmental Microbiology,* vol 43 (1982), p 1196.

Eligwe, C.A., "Microbial desulphurization of coal," *Fuel,* vol 67 (1988), p 451.

Hayes, T.D., W.J. Jewell, and R.M. Kabrick, "Heavy metal removal from sludges using combined biological/chemical treatment," *Proceedings of the 34th Industrial Waste Conference, Purdue University,* West Lafayette, IN, May 8-10, 1979 (Ann Arbor Science Publishers, Ann Arbor, MI, 1980), pp 529-543.

Hughes, M.N., and R.K. Poole, *Metals and Microorganisms* (Chapman and Hall, London, 1989).

Hutchins, S.R., M.S. Davison, J.A. Brierley, and C.L. Brierley, "Microorganisms in reclamation of metals," *Annual Review of Microbiology*, vol 40 (1986), p 311.

Karavaiko, G.I., "Microbiological processes for the leaching of metals from ores," *United Nations Environmental Programme* (Center for International Project, Moscow, USSR, 1985), p 68.

Kelly, D.P., and C.A. Jones, "Factors affecting metabolism and ferrous iron oxidation in suspensions and batch cultures of *Thiobacillus ferrooxidans*: relevance to ferric iron leach solution regeneration," *Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena,* L.E. Murr, A.E. Torma, and J.A. Brierley, eds. (Academic Press, New York, 1978).

Kiff, R.J., and S. Brown, "The development of an oxidative acid hydrolysis process for sewage sludge detoxification," *Proceedings International Conference on Heavy Metals in the Environment* (Amsterdam, September 1981), p 159.

Konishi, Y., H. Kubo, and S. Asai, "Bioleaching of zinc sulfide concentrate by *Thiobacillus ferrooxidans*," *Biotechnology and Bioengineering,* vol 39 (1991), p 66.

LeRoux, N.W., D.S. Wakerley, and S.D. Hunt, "Thermophilic thiobacillus-type bacteria from Icelandic thermal areas," *Journal of General Microbiology*, vol 100 (1977), p 197.

Lundgren, D.G., and M. Silver, "Ore leaching by bacteria," *Annual Review of Microbiology,* vol 34 (1980), p 263.

Murr, L.E., "Theory and practice of copper sulphide leaching in dumps and *in situ*," *Mineral Science Engineering,* vol 12 (1980), p 121.

Olver, J.W., W.C. Kreye, and P.H. King, "Heavy metal release by chlorine oxidation of sludge," *Journal Water Pollution Control Federation*, vol 47 (1975), p 2490.

Razzell, W.E., and P.C. Trussel, "Isolation and properties of an iron-oxidizing *Thiobacillus*," *Journal of Bacteriology,* vol 85 (1963), p 595.

Silverman, M.P., "Biological and organic chemical decomposition of silicates," *Biogeochemical Cycling of Mineral-forming Elements*, P.A. Trudinger and D.J. Swaine, eds. (Elsevier, Amsterdam, 1979), pp 445-465.

Smith, E.E., and K.S. Shumate, "The sulphide to sulphate reaction mechanism; a study of the sulphide to sulphate reaction mechanism as it relates to the formation of acid mine waters," *Water Pollution Control Research Series* (Ohio State University Research Foundation, Columbus, OH, 1970).

Sreekrishnan, T.R., R.D. Tyagi, J.F. Blais, and P.G.C. Campbell, "Kinetics of heavy metal bioleaching from sewage sludge: I. Effects of process parameters," *Water Research,* vol 27 (1993), p 1641.

Sugio, T., C. Domatsu, O. Munakata, T. Tano, and K. Lmai, "Role of a ferric ion-reducing system in sulphur oxidation of *Thiobacillus ferrooxidans*," *Applied and Environmental Microbiology,* vol 49 (1985), p 1401.

Torma, A.E., "Microbiological oxidation of synthetic cobalt, nickel and zinc sulfides by *Thiobacillus ferrooxidans*," *Revue Canadienne de Biologie,* vol 30 (1971), p 209.

Torma, A.E., and H. Sakaguchi, "Relation between the solubility product and the rate of metal sulphide oxidation by *Thiobacillus ferrooxidans*," *Journal of Fermentation Technology,* vol 56 (1978), p 173.

Tributsh, H., and J.C. Bennett, "Semiconductor-electrochemical aspects of bacterial leaching: I. Oxidation of metal sulphides with large energy gaps," *Journal of Chemical Technology and Biotechnology,* vol 31 (1981), p 565.

Tyagi, R.D., D. Couillard, and F. Tran, "Heavy metals removal from anaerobically digested sludge by chemical and microbiological methods," *Environmental Pollution*, vol 50 (1988), p 295.

Tyagi, R.D., T.R. Sreekrishnan, P.G.C. Campbell, and J.F. Balis, "Kinetics of heavy metal bioleaching from sewage sludge: II. Mathematical model," *Water Research*, vol 27 (1993), p 1653.

USEPA, "National sewage sludge survey: availability of information and data, and anticipated impacts on proposed regulations, proposed rule," *Federal Register,* vol 55 (1990), p 47209.

Vestal, J.R., and D.G. Lundgren, "The sulphite oxidation of *Thiobacillus ferrooxidans*," *Canadian Journal of Biochemistry,* vol 49 (1971), p 1125.

Wong, L., and J.G. Henry, "Bacterial leaching of heavy metals from anaerobically digested sewage sludge," *Water Pollution Research in Canada*, vol 18 (1983a), p 151.

Wong, L., and J.G. Henry, "Biological removal and chemical recovery of metals from sludges," *The 39th Annual Purdue Industrial Waste Conference* (Purdue University, May 8-10, 1983b), p 515.

Wong, L., and J.G. Henry, "Decontaminating biological sludge for agricultural use," *Water Science and Technology,* vol 17 (1984), p 575.

Uncited References

Brooks, C.S., "Metal recovery from industrial wastes," *Journal of Metals* (July 1986), p 50.

Bruce, A.M., and R.D. Davis, "Sewage sludge disposal: current and future options," *Water Science and Technology*, vol 21 (1989), p 1113.

Bryner, L.C., and R. Anderson, "Microorganisms in leaching sulfide minerals," *Industrial and Engineering Chemistry*, vol 49 (1957), p 1721.

Couillard, D., and G. Mercier, "Bacterial leaching of heavy metals from sewage sludge—bioreactors comparison," *Environmental Pollution*, vol 66 (1990), p 253.

Couillard, D., and G. Mercier, "Removal of metals and fate of N and P in the bacterial leaching of aerobically digested sewage sludge," *Water Research,* vol 27 (1993), p 1227.

Harrison, A.P., "The acidophilic Thiobacilli and other acidophilic bacteria that share their habitat," *Annual Review of Microbiology*, vol 38 (1984), p 265.

Irving, H., and R.J.P. Williams, "Order of stability of metal complexes," *Nature*, vol 162 (1948), p 746.

Jenkins, R.L., B.J. Scheybeler, M.L. Smith, R. Baird, M.P. Lo, and R.T. Huang, "Metals removal and recovery from municipal sludge," *Journal of Water Pollution Control Federation,* vol 53 (1981), p 25.

Lake, D.L., P.W.W. Kirk, and J.N. Lester, "Fractionation, characterization, and speciation of heavy metals in sewage sludge and sludge-amended soils: a review," *Journal of Environmental Quality,* vol 13 (1984), p 175.

Lanouette, K.H., "Heavy metals removal," *Chemical Engineering (Deskbook Issue)* (October 1977), p 73.

Markosyan, G.E., "A new acidophilic iron bacterium *Leptosprillum ferrooxidans*," *Biologicheskii Zhurnal Armenii*, vol 25 (1972), p 26.

Oake, R.J., C.S. Booker, and R.D. Davis, "Fractionation of heavy metals in sewage sludges," *Water Science and Technology*, vol 17 (1984), p 587.

Olson, G.J., F.D. Porter, J. Rubenstein, and S. Silver, "Mercuric reductase from a mercury-volatizing strain of *Thiobacillus ferrooxidans*," *Journal of Bacteriology,* vol 151 (1982), p 1230.

Razzell, W.E., and P.C. Trussell, "Microbiological leaching of metallic sulfides," *Applied Microbiology*, vol 11 (1963), p 105.

Sanmugasunderam, V., R.M.R. Branion, and D.W. Duncan, "A growth model for the continuous microbiological leaching of a zinc sulfide concentrate by *Thiobacillus ferrooxidans*," *Biotechnology and Bioengineering*, vol 22 (1985), p 1173.

Scheltinga, D.M.J., "Sludge in agriculture: the European approach," *Water Science and Technology*, vol 19 (1987), p 9.

Schmidtke, N.W., "Sludge generation, handling and disposal at phosphorus control facilities in Ontario," *Proceedings of the 2nd European Symposium on Characterization, Treatment and Use of Sewage Sludge* (Vienna, Austria, October 20-24, 1980).

Sommers, L.E., "Chemical composition of sewage sludges and analysis of their potential use as fertilizers," *Journal of Environmental Quality*, vol 6 (1977), p 225.

Sreekrishnan, T.R., R.D. Tyagi, J.F. Blais, and P.G.C. Campbell, "Kinetics of heavy metal bioleaching from sewage sludge: I. Effects of process parameters," *Water Research*, vol 27 (1993), p 1641.

Torma, A.E., C.C. Walden, and R.M.R. Branion, "Microbiological leaching of a zinc sulfide concentrate," *Biotechnology and Bioengineering*, vol 12 (1970), p 501.

Torma, A.E., C.C. Walden, and R.M.R. Branion, "The effect of carbon dioxide and particle surface area on the microbiological leaching of a zinc sulfide concentrate," *Biotechnology and Bioengineering*, vol 14 (1972), p 777.

Tuovinen, O.H., S.I. Niemila, and H.G. Gylenberg, "Tolerance of *Thiobacillus ferrooxidans* to some metals," *Antonie van Leeuwenhoek J. Microbiol. Serol.*, vol 37 (1971), p 489.

Tyagi, R.D., D. Couillard, and F. Tran, "Studies on microbial leaching of heavy metals from municipal sludge," *Water Research*, vol 22 (1990), p 229.

Tyagi, R.D., J. Blais, J. Aucalir, and N. Meunier, "Bacterial leaching of toxic metals from municipal sludge: influence of sludge characteristics," *Water Environment Research*, vol 65 (1993), p 196.

Wong, J.G., "Fate of indicator organisms in sludge during bacterial leaching of metals," *Canadian Journal of Civil Engineering*, vol 18 (1991), p 1.

Wozniak, D.J., and J.Y.C. Huang, "Variables affecting metal removal from sludge," *Journal of Water Pollution Control Federation*, vol 54 (1982), p 1574.

3 Microbial Sorption of Heavy Metals

Microbiological methods are increasingly applied to the removal and/or recovery of metal ions from aqueous solutions. These applications vary from large scale processes such as the removal of heavy metals from industrial effluents and mine leachate to much smaller operations involving the recovery of precious metals from process streams or wastewater from the electroplating and jewelry industries (Hughes and Poole 1989). Intact microbial cells, living or dead, and derived microbial products can be highly efficient bioaccumulators of both soluble and particulate forms of metals, especially from dilute solutions (Shumate and Strandberg 1985).

Metal removal or recovery from solution may involve the following pathways:

- the binding of metal cations to cell surfaces
- transport of the metal into the cell, possibly by active transport
- formation of metal-containing precipitates by reaction with extracellular polymers or microbially produced anions such as sulfide or phosphate.

In addition, processes have been developed using immobilized extracellular materials and cellular ligands.

With increasing awareness of the many physicochemical interactions that can occur in nonmetabolic uptake of metal ions by microbial biomass, the term "biosorption" is widely used to describe nondirected physicochemical reactions that occur between metal species and cellular components (Shumate and Strandberg 1985).

Binding of Metals to Cell Surfaces

Cell surfaces are negatively charged because of the presence of ionized groups such as carboxylate, hydroxyl, and phosphate in the various cell wall polymers. The extent of uptake of metal may vary with pH because of the protonation of these anionic groups. Organisms have various distributions of charge and geometry for these ionized binding groups, possibly resulting in selective binding of certain metal ions.

Metal-accumulative bioprocesses are generally divided into two categories: biosorptive uptake by nonliving, nongrowing biomass or biomass products and bioaccumulation by living cells. The relative merits and disadvantages of each are summarized in the Tables 1 and 2 (Macaskie 1990). The major advantage of using dead cells is that they do not require the addition of nutrient materials and there is no need to protect them against contamination by other organisms. Various types of waste biomass are easily available at low or no cost.

The use of a process involving growing cultures of organisms is more complex. They may, for example, be sensitive to the toxic effects of the metals they accumulate; in this instance, resistant strains are required. However, metal-resistant cells sometimes function by preventing the transport of toxic metal ions into the cell by binding them tightly on the surface; these strains may be ideal for use in small-volume metal recovery applications.

Immobilization or Aggregation of Cells

Cells may be aggregated into stable particles by cross-linking with a bifunctional reagent such as glutaraldehyde, by use of flocculating agents, or by heat or acid treatment. Filamentous fungus *Aspergillus niger* can be readily grown as pellets 4 mm in diameter by adjustment of pH. Heat-killed pellets retain their physical structure and adsorptive capacity over long periods of time. Metal-sorbing bacteria, *Zoogloea ramigera,* may be flocculated by adjustment of the pH of the medium to 5.5 (Norberg and Persson 1984).

Cell-immobilization techniques involve adsorbing cells to surfaces such as silica gel, metal oxides, porous glass, or ceramic material with accurately known pore sizes.

Table 1. Advantages and disadvantages of metals adsorption by immobilized nonliving biomass.

Advantages	Disadvantages
• Growth-independent, nonliving biomass, not subject to toxicity limitations. No necessity for nutrients in feed solution, or problems of disposal of surplus nutrients or metabolic products. • Process not governed by physiological constraints. • Choice of immobilization technique not governed by toxicity limitations or thermal inactivation. • Very rapid and efficient metal uptake; the biomass behaves as an ion exchanger. • Metals can be desorbed readily, and recovered. • The system can be mathematically defined.	• Early saturation: when metal-interactive sites are occupied, metal desorption is necessary prior to further use, irrespective of the metal value. • Adsorptive uptake is sensitive to pH, metal speciation. • There is no potential for biologically altering the metal valency state, e.g., to give less soluble forms. • There is no potential for degradation of organometallic species. • The potential for biological process improvement is limited since the cells are not metabolizing; production of adsorptive agent occurs during pregrowth.

Table 2. Advatages and disadvantages of metals adsorption by immobilized living cells.

Advantages	Disadvantages
• Although each cell may become saturated , the system as a whole is self-replenishing due to growth. • The metal is deposited in an altered chemical state and is less sensitive to spontaneous desorption. • Metabolic activity may be the only economic way to achieve changes in valency state or degrade. • There is the potential for mutant isolation or genetic manipulation to improve the strain, since it is a microbial property rather than a product under exploitation. • Two or more organisms can be employed synergistically.	• Toxicity; often the metal can be presented only at low concentrations, but metal-resistant strains have been used. • Necessity to prevent the flow under physiologically permissive conditions. • Need for growth nutrients. • Disposal of metabolic products and unconsumed growth nutrients. • Metabolic products may form complexes with metals to retain them in solution. • Potential for desorptive metal recovery is limited since metals may be intracellularly bound. • Difficulties in mathematical modeling of a non-defined system.

However, the most common immobilized technique is trapping cells in polymers such as K carrageenan, calcium alginate, and cellulose acetate.

Criteria for Surface-Binding Processes

The following criteria need to be satisfied if a metal-recovery process involving surface binding to microorganisms is to be commercially viable (Hughes and Poole 1989):

- organism must be immobilized or granulated readily
- it must have a metal removal efficiency over 99 percent
- it must have high extracellular binding capacity
- it must be effective over a wide pH range
- it must be unaffected by at least 100 recycles, following acid or ligand treatment
- the process must be cheaper than ion exchange or solvent extraction methods.

Binding of Metals to Gram-Positive Bacteria

The main structural compound of Gram-positive bacterial walls is peptidoglycan, which is a polymer made up of carbohydrate strands (a repeated dimer of N-acetylglucosamine and N-acetylmuramic acid) cross-linked by a small peptide. These peptides contain glutamic acid residues; the carboxylate side chains play an important role in metal-binding. Beveridge and Murray (1980) found that the capacity of *Bacillus subtilis* walls to bind Osmium (III) is reduced dramatically if the carboxylate group is esterified; they reported that 55 percent of cell walls from *B. subtilis* consisted

of teichoic acid. Teichoic acid is a linear polymer of glycerol phosphate linked to peptidoglycan. Monovalent cations bind to phosphodiester groups of teichoic acid on a 1:1 basis; divalent cations bridge adjacent phosphate groups and polymer strands. Other organisms may contain more complex teichoic acids, such as polyribitol teichoic acid, which lead to different metal-binding properties (Hughes and Poole 1989).

Multiple layers of peptidoglycan molecules in the cell wall give a porous open structure that results in an enhanced surface area for sorption. In addition to electrochemical binding, the porous structure of the wall allows the build-up of substantial amounts of precipitated metals. This allows for the binding of greater amounts of metals than allowed by the stoichiometry of the binding groups in *B. subtilis* walls (Beveridge and Murray 1976).

Sahoo et al. (1991) found that biomass (1.48 to 1.52 g dry weigh per liter) obtained from polysaccharide-producing *B. circulans* culture removed 80 percent of copper and 44 percent of cadmium from solutions containing 495 ppm copper and 492 ppm cadmium, respectively. The pH of the metal solutions had a pronounced effect on the metal-accumulating capacity of the organism.

Mullen et al. (1989) examined four bacteria, *B. cereus*, *B. subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* for the ability to remove Ag^{+}, Cd^{+2}, Cu^{+2}, and La^{+3} from solution; they found 89 percent of the total Ag^{+} from the 1 mM solution, and only 12, 29, and 27 percent of the total Cd^{+2}, Cu^{+2}, and La^{+3} removal, respectively. Electron microscopy indicated that La^{+3} accumulated at the cell surface as needle-like, crystalline precipitates, and silver precipitated as discrete colloidal aggregates at the cell surface. Neither Cd^{+2} nor Cu^{+2} provided enough electron scattering to identify the location of sorption. Affinity for bacterial removal of these metals decreased in the following order Ag > La > Cu > Cd.

Binding of Metals to Gram-Negative Bacteria

Cell walls of Gram-negative bacteria are made up of two membrane bilayers. The outer membrane is linked chemically to a peptidoglycan monolayer that lies between the outer and plasma membranes. Gram-negative bacteria bind about one-tenth of the amount of metal bound by the Gram-positive bacteria.

Beveridge and Fyfe (1985) investigated the binding of metal ions to the purified cell envelope of *E. coli* K12 by suspending the envelopes in 5 mM metal solutions for 10 minutes at 23 °C. Beveridge and Fyfe (1985) observed that the envelopes bound large amounts of Hf and Os (>0.9 μmol/mg dry weight), intermediate amounts of Pb, Zn, Zr, Fe (III), Mn, Mo, Mg, Co (II), and Ce (IV) (0.1 to 0.4 μmol/mg dry weight) and smaller

amounts of other metal cations. Electron microscopy indicates that most metal deposition occurred at the polar head groups of the membrane constituents and on the peptidoglycan.

Uptake of Metals by Fungi and Yeasts

Fungi and yeasts remove aqueous metals by binding to the cell surface and by intracellular transport (Hughes and Poole 1989). Living fungal cells thus accumulate higher levels of metal than dead cells. The main structural components of fungal cell walls are chitin and chitosan, which are polymers of N-acetylated and nonacetylated glucosamine, respectively; the latter is the more effective ligand. Other ligand groups are phosphate, carboxyl, amine, and hydroxyl, plus groups in melanin and other pigments (Strandberg, Shumate, and Parrott 1981).

Melanin-pigmented *Aureobasidium pullulans* sorbed higher amounts of heavy metals than nonpigmented cells. Gadd and de Rome (1988) have demonstrated that fungal melanins obtained from fungal species, *A. pullulans* and *Cladosporium resinae*, have a higher affinity for Cu^{+2} than cells themselves. Uptake capacities of melanin-pigmented cells were greater than those of nonpigmented cells, which further validates that melanin plays a significant role in the uptake of metals by fungal cell walls. In addition to an application in metal biosorption, melanin plays a significant role in the survival of organisms in a metal-rich medium by reducing the concentration of potentially toxic metal ions in the solution.

Strandberg, Shumate, and Parrott (1981) reported that uranium accumulated on the surfaces of *Saccharomyces cerevisiae* cells; they found that the surface-associated accumulation of uranium by *S. cerevisiae* was consistent with the hypothesis that metal biosorption occurs as a result of the interaction of positively charged metal ions with negatively charged reactive sites ($R\text{-}COO^{-}$, PO_4^{-2}) on cell surfaces and on extracellular polymers. The rate and extent of accumulation were subject to environmental parameters, such as pH, temperature, and the presence of certain anions and cations. Environmental parameters not only affect reactive metal-binding sites, but they also influence the solution chemistry of uranium. In the pH range of the optimum uranium uptake (pH 3.0 to 4.5), soluble uranium exists as UO_2^{+2} and hydrolysis products such as $(UO_2)_2(OH)_2^{+2}$, $UO_2(OH)^{+}$, and $(UO_2)_3(OH)_5^{+}$. The attempts by Strandberg, Shumate, and Parrott (1981) to determine the chemical state of uranium as it exists on cell surfaces was unsuccessful. However, they observed that, as uranium is taken up by cells, the pH increased from about 4.0 to 6.0, indicating a release of free hydroxyl ions. This suggested that the UO_2^{+2} ion was the predominant form of the bound uranium. Strandberg, Shumate, and Parrott (1981) indicated that

uranium could be removed chemically from *S. cerevisiae* cells, and the cells could then be reused as a biosorbent.

Volesky, May, and Holan (1993) examined cadmium uptake by nonliving and resting cells of yeast, S. *cerevisiae,* obtained from aerobic and anaerobic cultures. The highest cadmium uptake exceeding 70 mg Cd/g was observed with aerobic baker's yeast biomass. A higher sorbing capacity was observed for resting cells when compared to dry, nonliving yeasts, which usually accumulate less than 20 mg Cd/g. Metal deposits in the resting cells localized exclusively in vacuoles indicate the possibility of a different metal-sequestering mechanism between the resting and nonliving cells. Uptake of cadmium was relatively fast because 75 percent of the sorption was completed in less than 5 minutes. The large-scale availability of yeast as waste biomass makes it attractive for industrial applications.

Wales and Sagar (1990) demonstrated the recovery of metal ions by microfungal filters. Many microfungi contain chitin and chitosan as an integral part of their cell wall structure. Binding of heavy metal ions by chitosan and partly deacetylated chitin is a direct consequence of the primary amine groups. This interaction is most effective for metals that form complexes with ammonia. Of the microfungi studied, hypae from *Mucor mucedo* and *Rhizomucor miehei* were found to be the most effective for sorption of metal ions following a treatment with hydroxide to expose the chitin and chitosan. Chemically treated mycelia was shown to bind silver, zinc, lead, copper, nickel, cobalt, cadmium, iron, and chromium.

Wales and Sagar (1990) prepared microfungal filters from mixed slurries of treated mycelia and various conventional paper-making and textile fibers. The resulting papers had exceptionally good tensile- and bursting-strength properties, particularly in the wet state. Wales and Sagar (1990) found the papers containing 1 g treated mycelia removed up to 90 percent of various metal ions in solutions at a flow rate of 0.5 $cm^3/cm^2/min$.

Binding of Metals by Algae

Kuyucak and Volesky (1989a) reported that certain seaweeds, particularly Phaeophyceae and Phodophyceae, are capable of accumulating metals from an aqueous environment because of their polysaccharide content, such as alginates and carrageenan. Kuyucak and Volesky (1989a) suggested two mechanisms of binding of radioactive elements by tropical marine algae: ion exchange and complex formation. Many studies on metal removal by algae have been performed with living organisms for environmental, toxicological, and pharmaceutical purposes rather than with

industrial applications in mind. More recently, attention has turned to the application of nonliving algae for the metal removal and recovery.

Kuyucak and Volesky (1989b) reported the screening and selection of the most potent marine algae for sequestering of cobalt. Various algae tested for their study are tabulated in Table 3.

All the biomass showed some degree of cobalt sorption; the brown algae, *Ascophyllum nodosum,* exhibited the highest cobalt uptake capacity (approximately 156 mg/g) and high affinity at low equilibrium Co^{+2} concentrations. A successful desorption of cobalt from the biomass by acidic $CaCl_2$ solutions indicated that the metal uptake is a reversible phenomenon, implying physical sorption of cobalt. Chemical and instrumental analysis including electron microscopy, infrared spectroscopy, X-ray dispersion, and diffraction analysis provided supporting evidence that the biosorption mechanism involves predominantly ion exchange (Kuyucak and Volesky 1989b).

Holan, Volesky, and Praseto (1993) studied the dried biomasses of nonliving brown marine algae (*Sargassum natans*, *Fucus vesiculosus*, and *Ascophyllum nodosum*) and found a high uptake of cadmium from aqueous solutions. Biomass of *A. nodosum* accumulated the highest amount of Cd, exceeding 100 mg/g (at the residual concentration of 100 mg Cd/L and pH 3.5); it outperformed a commercial ion exchange resin. A new biosorbent material based on *A. nodosum* biomass was prepared by reinforcing the algal biomass with formaldehyde cross-linking. The prepared sorbent possessed good mechanical properties, chemical stability of the cell wall polysaccharides, and low swelling volume. Desorption of deposited cadmium with 0.1 to 0.5 M HCl did not change the metal uptake capacity of biosorbent through five adsorption/desorption cycles.

Garnham, Geoffrey, and Gadd (1992) examined the accumulation of Co, Zn, and Mn by *Chlorella salina* immobilized in calcium alginate microbeads using the radioisotopes ^{60}Co, ^{54}Mn, and ^{65}Zn. They found a rapid biosorption of the metals to

Table 3. Biomass samples of algae and fungi.

Common Name	Scientific Name
red algae	*Porphyra tenera, Palmaria palmata, Chondrus crispus*
brown algae	*Sargassum natans, Undra* sp., *Ascophyllum nodosum, Macrocystis pyrifera, Laminaria* sp.
green algae	*Halimeda opuntia, Codium taylori*
fungal biomass	*Aspergillus niger, Rhizopus arrhizus.*

C. salina cell walls and the alginate matrix followed by a slower energy-dependent phase of uptake. Under similar conditions, immobilized cells accumulated greater amounts of Co, Zn, or Mn than free cells.

Darnell et al. (1986a) observed that pH dependence of Au^{+3}, Ag^{+}, and Hg^{+2} binding to the algae *C. vulgaris* was different than that of the other metals. Between pH 5 and 7, a variety of metal ions sorbed strongly to the cell surface, and most of these algal-bound metal ions were desorbed by lowering the pH to 2, except Au^{+3}, Ag^{+}, and Hg^{+2}; these latter ions remained strongly bound even at pH 2. The addition of a strong ligand was required to elute these metals from the algal surface. Darnell et al. (1986b) found that algal-bound gold and mercury can be selectively eluted by using mercapto-ethanol; and they also demonstrated various elution schemes for the selective recovery of Cu^{+2} and Zn^{+2} from an equimolar mixture.

Apart from a continuing search for a new potent biosorbent, developing biosorbent materials that could be used in continuous-flow sorption columns as well as the scale-up parameters for full-scale sorption process requires further works. To be cost effective in competition with established ion exchange resins, new biosorbent materials need to be effective metal binders and perform efficiently under various process conditions.

Immobilization of Metals by Extracellular Compounds: Removal of Metals From Wastewater in the Activated Sludge Process

Many microorganisms produce negatively charged extracellular polymers (ECP) that enable them to grow in their natural habitat as flocs or biofilms. ECPs form a matrix around the cell surface and create a protective buffer between the surrounding environment and the cell (Brown and Lester 1979). The matrix layer also accumulates growth-limiting nutrients from the surrounding environment for subsequent transport into the cells (Rudd, Sterritt, and Lester 1983). This anionic polysaccharide material binds metal ions effectively and has been implicated in the binding and precipitation of heavy metals by sewage sludge.

Many studies found that various species of bacteria isolated from activated sludge can produce extracellular polymers (Dugan and Pickrum 1972; Brown and Lester 1979; Rudd, Sterritt, and Lester 1983). These ECPs, extracted from activated sludge, mainly consisted of polysaccharide, proteins, and nucleic acids (Brown and Lester 1979). Morgan, Forster, and Evison (1990) used the thermal extraction/solvent precipitation techniques to extract ECPs from several different activated sludges and found that the ECPs concentration ranged from 10.4 to 90.2 mg/g suspended solids.

Factors that affect the concentrations of ECPs produced in bacterial cultures and activated sludge have been identified as ratios in the growth medium (i.e., C:N, C:P, and C:S), amount of dissolved O_2, biological oxygen demand, and biomass loading rates. Capsular materials produced under conditions of acute phosphorous limitation increased the metal-binding capacity of sludge organisms (Casey and Wu 1977).

Brown and Lester (1982) reported that *Klebsiella aerogenes* produced extracellular polymers and loose slime polymers, which can be extracted readily. Comparisons between the binding capacities of intact biomass, stripped biomass and extracted polymers clearly showed that the ECP is responsible for the binding and precipitation of added metals. Brown and Lester (1982) showed that inactivation of activated sludge cells had little effect on the uptake of various metals; this confirms that the metal uptake is a passive process. However, loss of viability caused a marked decrease in the uptake of nickel, suggesting that some of the nickel was removed by intracellular accumulation.

AlgaSORB®

Bio-recovery Systems, Inc.,* has developed a proprietary, algal-based material, called "AlgaSORB," which can be used on a commercial basis to remove and recover heavy metal ions from point-source industrial wastewater, contaminated groundwaters, or mining process streams (Darnell 1991). AlgaSORB is packed into columns through which water containing heavy metal ions is flushed. When harvested, natural algal cells are packed into columns, and the cells tend to aggregate and form clumps. These clumps are difficult to force water through, even under high pressures. AlgaSORB alleviates this difficulty by immobilizing the cells into a polymeric matrix. Because cells are killed in the immobilization process, the algal matrix will not be affected by conditions that would normally kill living cells. Darnell et al. (1986a) reported that the immobilized process serves two purposes:

- It protects the algal cells from decomposition. AlgaSORB immersed in aqueous solution for over 2 years has shown no decrease in metal-binding efficiency.
- It produces a hard material that can be packed into columns.

* Bio-recovery Systems, Inc., 2001-T Copper Ave., Las Cruces, NM 88005-7105, Phone (505)523-0405, Fax (505)523-1638.

Darnell (1991) indicated that AlgaSORB functions as biological ion-exchange resins. The metal ions can be sorbed and desorbed over many cycles without noticeable losses in efficiency. In contrast to current ion-exchange technology, divalent cations (Ca^{+2} and Mg^{+2}) and monovalent cations (Na^{+} and K^{+}) do not significantly interfere with the binding of heavy metal ions by the algal matrix. Darnell (1991) demonstrated that calcium or magnesium ion concentrations as high as 10,000 mg/L have little or no effect on AlgaSORB sorption of copper at concentrations as low as 6.5 mg/L. Because calcium and magnesium ions frequently are present in high concentrations and compete for sorption sites, sorptions of Ca^{+2} and Mg^{+2} to ion-exchange resins often limits the performance of ion exchange. Thus, AlgaSORB has the potential to be particularly useful for removing metal ions from hard waters.

Cited References

Barkley, N.P., "Extraction of mercury from groundwater using immobilized algae," *Journal of the Air and Waste Management Association*, vol 41 (1991), p 1387.

Beveridge, T.J., and W.S. Fyfe, "Metal fixation by bacterial cell walls," *Canadian Journal of Earth Sciences*, vol 22 (1985), p 1893.

Beveridge, T.J., and R.G.E. Murray, "Uptake and retention of metals by cell walls of *Bacillus subtilis*," *Journal of Bacteriology*, vol 127 (1976), p 1502.

Beveridge, T.J., and R.G.E. Murray, "Sites of metal deposition in the cell wall of *Bacillus subtilis*," *Journal of Bacteriolgy*, vol 141 (1980), p 876.

Brown, M.J., and J.N. Lester, "Metal removal in activated sludge: the role of bacterial extracellular polymers," *Water Research*, vol 13 (1979), p 817.

Brown, M.J., and J.N. Lester, "Comparison of bacterial extracellular polymer extraction methods," *Applied Environmental Microbiology*, vol 40 (1980), p 179.

Brown, M.J., and J.N. Lester, "Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge: effects of metal concentration," *Water Research*, vol 16 (1982), p 1548.

Casey, J.D., and Y.C. Wu, "Removal of copper and cadmium by metabolically controlled activated sludge," *Proceedings of the 32nd Industrial Waste Conference*, May 10-11, 1977, Purdue University, West Lafayette, IN (Ann Arbor Science Publishers Inc., Ann Arbor, MI, 1978), pp 141-152.

Darnell, D.W., B. Greene, M.T. Henzi, J.M. Hosea, and R.A. McPherson, "Recovery of heavy metal ions by immobilized algae," *Trace Metal Removal From Aqueous Solutions*, R. Thompson, ed., Special Publication No. 61 (London: Royal Society of Chemistry, 1986a), pp 1-24.

Darnell, D.W., B. Greene, M.T. Henzi, J.M. Hosea, and R.A. McPherson, "Selective recovery of gold and other metal ions from an algal biomass," *Environmental Science & Technology,* vol 20 (1986b), p 206.

Darnell, D.W., "Removal and recovery of heavy metal ions from wastewaters using a new biosorbent; AlgaSORB," *Biological Processes, vol. 3, Innovative Hazardous Waste Treatment Technology Series,* H.M. Freeman, ed. (1991), pp 65-72.

Dugan, P.R., and H.M. Pickrum, "Removal of mineral ions from water by microbially produced polymers," *Proceedings of the 27th Industrial Waste Conference, Part 2,* May 2-4, 1972 (Purdue University, West Lafayette, IN, 1972), pp 1019-1038.

Gadd, G.M., and L. de Rome, "Biosorption of copper by fungal melanin," *Applied Microbiology and Biotechnology,* vol 29 (1988), p 610.

Garnham, G.W., A.C. Geoffrey, and G.M. Gadd, "Accumulation of cobalt, zinc and manganese by the estuarine green microalga *Chlorella salina* immobilized in aliginate microbeads," *Environmental Science & Technology,* vol 26 (1992), p 1764.

Holan, Z.R., B. Volesky, and I. Prasetyo, "Biosorption of cadmium by biomass of marine algae," *Biotechnology and Bioengineering,* vol 41 (1993), p 819.

Hughes, M.N., and R.K. Poole, *Metals and Micro-organisms* (Chapman and Hall, London, 1989).

Hutchins, S.R., M.S. Davison, J.A. Brierly, and C.L. Brierly, "Micro-organisms in reclamation of metals," *Annual Review of Microbiology,* vol 40 (1986), p 311.

Kuyucak, N., and B. Volesky, "Accumulation of cobalt by marine alga," *Biotechnology and Bioengineering,* vol 33 (1989a), p 809.

Kuyucak, N., and B. Volesky, "The mechanism of cobalt biosorption. I," *Biotechnology and Bioengineering,* vol 33 (1989b), p 823.

Macaskie, L.E., "An immobilized cell bioprocess for the removal of heavy metals from aqueous flows," *Journal of Chemical Technology and Biotechnology,* vol 49 (1990), p 357.

Morgan, J.W., C.F. Forster, and L. Evison, "A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges," *Water Research,* vol 24 (1990), p 743.

Norberg, A.B., and H. Persson, "Accumulation of heavy-metal ions by *Zoogloea ramigera,*" *Biotechnology and Bioengineering,* vol 26 (1984), p 239.

Rudd, T., R.M. Sterritt, and J.N. Lester, "Stability constant and complexation capacities of complexes formed between heavy metals and extracellular polymers from activated sludge," *Journal of Chemical Technology and Biotechnology,* vol 33 (1983), p 374.

Sahoo, D.K., R.N. Kar, and R.P. Das, "Bioaccumulation of heavy metal ions by *Bacillus circulans,*" *Bioresource Technology,* vol 41 (1992), p 177.

Shumate, S.E., and G.W. Strandberg, "Accumulation of metals by microbial cells," *Comprehensive Biotechnology, Vol. 4*, M. Moo-Young, C.N. Robinson, and J.A. Howell, eds. (Pergamon Press, New York, 1985).

Strandberg, G.W., S.E. Shumate, and J.R. Parrott, "Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*," *Applied Environmental Microbiology*, vol 41 (1981), p 237.

USEPA, *Emerging Technologies: Bio-recovery Systems Removal and Recovery of Metal Ions From Groundwater*, Report No. 540/5-90/005a. (USEPA, Cincinnati, OH, August 1990).

Volesky, B., H. May, and Z.R. Holan, "Cadmium biosorption by *Saccharomyces cerevisiae*," *Biotechnology and Bioengineering*, vol 41 (1993), p 826.

Wales, D.S., and B.F. Sagar, "Recovery of metal ions by microfungal filters," *Journal of Chemical Technology and Biotechnology*, vol 49 (1990), p 345.

Uncited References

Cheng, M.H., J.W. Patterson, and R.A. Minear, "Heavy metal uptake by activated sludge," *Journal of Water Pollution Control Federation,* vol 47 (1975), p 362.

Ferguson, J., and B. Bubela, "The Concentration of Cu (II), Pb (II) and Zn (II) from aqueous solutions by particulate algae matter," *Chemical Geology*, vol 13 (1974), p 163.

Gourdon, R., E. Rus, S. Bhende, and S.S. Sofer, "A comparative study of cadmium uptake by free and immobilized cells from activated sludge," *Journal of Environmental Science and Health* Part A, vol 25, No. 8 (1990), p 1019.

Horikosshi, T., A. Nakajima, and T. Sakaguchi, "Studies on the accumulation of heavy metal elements in biological systems," *European Journal of Applied Microbiology and Biotechnology*, vol 12 (1981), p 90.

Kodukula, P.S., "Distribution of Cadmium and Nickel Between Soluble and Solid Phases of Activated Sludge," Ph.D. Dissertation, Illinois Institute of Technology (1984).

Kurek, E., J. Czaban, and J. Bollag, "Sorption of cadmium by microorganisms in competition with other soil constituents," *Applied Environmental Microbiology*, vol 43 (1982), p 1011.

Lawson, P.S., R.M. Sterritt, and J.N. Lester, "Adsorption and complexation mechanisms of heavy metal uptake in activated sludge," *Journal of Chemical Technology and Biotechnology,* vol 34 (1984), p 253.

Mullen, M.D., D.C. Wolf, F.G. Ferris, T.J. Beveridge, C.A. Flemming, and G.W. Bailey, "Bacterial sorption of heavy metals," *Applied Environmental Microbiology,* vol 55 (1989), p 3143.

Okieimen, F.E., and J.N. Okundayne, "Removal of cadmium and copper ions from aqueous solutions with thiolated maize (*Zea mays*) cob meal," *Biological Wastes*, vol 30 (1989), p 225.

Parkinson, G., "Metals recovery makes big splash in Canada," *Chemical Engineering* (September 1985), p 19.

Rudd, T., R.M. Steritt, and J.N. Lester, "Mass balance of heavy metal uptake by encapsulated culture of *Klebsiella aerogenes*," *Microbial Ecology*, vol 9 (1983), p 261.

Scott, J.A., and S.J. Palmer, "Cadmium bio-sorption by bacterial exopolysaccharide," *Biotechnology Letters*, vol 10 (1988), p 21.

Shuttleworth, K.L., and R.F. Unz, "Sorption of heavy metals to the filamentous bacterium *Thiothrix* strain A1," *Applied Environmental Microbiology*, vol 59 (1993), p 1274.

Tsezos, M., and A.A. Deutschmann, "An investigation of engineering parameters for the use of immobilized biomass particles in biosorption," *Journal of Chemical Technology and Biotechnology*, vol 48 (1990), p 29.

4 Microbial Precipitation of Heavy Metals Under Sulfate-Reducing Conditions

Microbial sulfate reduction has been identified as a valuable biological process for removing inorganic contaminants from wastewater (Wakao et al. 1979; Dvorak et al. 1992). Sulfate-reducing bacteria (SRB), under anaerobic conditions, oxidize simple organic compounds (such as acetic acid and lactic acid) with sulfate and generate hydrogen sulfide and bicarbonate ion:

$$SO_4^{-2} + CH_3COOH \rightarrow H_2S + 2\,HCO_3^- \quad (10)$$

$$3\,SO_4^{-2} + 2\,\underset{\text{(lactic acid)}}{CH_3CH(OH)COOH} \rightarrow 3\,H_2O + 6\,HCO_3^- \quad (11)$$

Hydrogen sulfide generated from the biological reaction reacts with heavy metal ions to form insoluble metal sulfides that easily can be separated from solution:

$$H_2S + M^{+2} \rightarrow MS_{(S)} + 2\,H^+ \quad (12)$$

where M includes any metal ion that can form insoluble metal sulfide. Bicarbonate ions produced during the SRB reaction buffer the solution pH to, typically, in the range of 6 to 7 (Dvorak et al. 1992). In the case of acidic wastewater, this buffering capacity also will cause some metal ions to precipitate as insoluble hydroxides or oxides:

$$Al^{+3} + 3\,H_2O \rightarrow Al(OH)_{3(s)} + 3\,H^+ \quad (13)$$

Sulfide Precipitation

Heavy metal removal from wastewater by sulfide precipitation is an efficient process because of the high reactivity of sulfides (S^{-2}, HS^-) with heavy metal ions and the insolubility of metal sulfides over a broad range of pH. In contrast, metal removal processes utilizing hydroxide precipitation require a narrow range of pH values because different metal hydroxides reach their minimum solubilities at different pH values. Moreover, hydroxide sludges have high water content and poor settleability. Rouse (1976) and Metzner (1977) reported that the treatment systems with sulfide precipitation generally achieve effluent concentrations of various metals in the low microgram per liter range, and the resulting effluent easily meets most effluent standards. Stumm and Morgan (1981) have demonstrated that, for the system associated with carbonate, extremely small partial pressures of H_2S are sufficient to convert metal carbonate into metal sulfide. The U.S. Environmental Protection Agency (1980) reported that low metal solubility can be achieved even in the presence of complexing and chelating agents. Drawbacks of sulfide precipitation are that sulfide is a hazardous chemical, and capital and operating costs for the sulfide process are about 10 percent higher than for conventional hydroxide system (Patterson 1985).

Sulfate-Reducing Bacteria

The dissimilatory sulfate reduction process is carried out by the so-called "sulfate-reducing organisms" that oxidize organic matter by utilizing the sulfate ion as an electron acceptor. Most of the reduced sulfur is released into the environment as the sulfide ion, but a small amount of reduced sulfur is assimilated by the organisms.

Sulfate-reducing bacteria (SRB) are strict anaerobes. Unlike the facultative denitrifiers, SRB do not have an alternative aerobic respiratory metabolic pathway (Stanier et al. 1986).

Desulfovibrio and *Desulfotomaculum* are the first two SRB identified. In recent years, several new genera (e.g., *Desulfobacter, Desulfonema,* and *Desulfobulbus)* have been identified. Selection of predominant SRB species depends on the carbon source. *Desulfovibrio, Desulfovonas, Desulfotomaculm,* and *Desulfobulus* utilize lactate, pyruvate, ethanol, or certain fatty acids as carbon and energy sources. *Desulfobacter, Desulfococcus, Desulfosarcina,* and *Desulfonema* specialize in the oxidation of fatty acids, particularly acetate.

Middleton and Lawrence (1977) reported that the synthesis of sulfate-reducing biomass is relatively low. They observed the specific yield (Y) value of 0.06 mg

volatilized suspended solids per milligram chemical oxygen demand, which is a typical value for anaerobes. Thimann (1963) indicated that the SRB were able to utilize only a limited number of organic compounds. Fermentative end products, such as lactate, ethanol, short-chain fatty acids, formate, and hydrogen produced from other anaerobes usually are the electron donors for sulfate reduction. The end products of organic carbon oxidation by SRB usually are acetate, water, and carbon dioxide. Organic compounds are oxidized only to acetate because most SRB do not possess the enzymes of the tricarboxylic acid (TCA) cycle, which is responsible for the complete oxidation of acetic acid to carbon dioxide (Stanier et al. 1986). *Desulfobacter* and *Desulfruomonas*, in contrast, have a complete TCA cycle and can oxidize acetate completely to carbon dioxide.

Middleton and Lawrence (1977) studied the kinetics of microbial sulfate reduction in a suspended-growth system, using acetate as a primary carbon source. They found that acetate utilizing SRB grown in continuous culture followed the monod-type kinetic growth model. The effect of temperature on the growth rate of SRB over the range of 21 to 31 °C was described by the Arrhenius-type relationship.

Postgate (1984) observed that the growth of SRB on ethanol was extremely poor but the yield of sulfide was high; this indicates that ethanol can support sulfate reduction but not growth. The use of ethanol may be advantageous to environmental applications because of less biomass production.

Biological Metal Sulfide Precipitation

Anaerobic sulfide reduction processes have been applied for sulfate removal from wastewater (Middleton and Lawrence 1977) and sulfur recovery (Maree and Strydom 1985). Maree and Strydom (1985) operated an upflow anaerobic filter for biological sulfate reduction, using glucose as an electron donor, and harvested sulfur from the biologically produced sulfide through a photosynthetic reaction.

Pugsley et al. (1970) studied the biological sulfate reduction process for neutralization of low pH (pH as low as 1.5) and removal of heavy metals from Rocky Mountain mine drainage in Western Colorado that contained considerable quantities of zinc, copper, manganese, aluminum, lead, cadmium, and arsenic, in addition to its high content of iron. Pugsley et al. (1970) reported that the activity of SRB partially neutralized the acidic liquid to about pH 3.5 and removed zinc, copper, and ferrous iron from liquid as metal sulfide precipitates. Pugsley et al. (1970) isolated cultures of *Desulfovibrio desulfuricans* that can grow in partially neutralized mine water and produce sufficient hydrogen sulfide for metal precipitation.

Murr, Torma, and Brierley (1978) reported that the pH of SRB culture medium strongly influences the specific growth rate and the rate of metal removal. The optimum pH for metal removal was observed to be 6.0, which agrees with the optimum pH for specific growth of SRB. In a continuous culture of SRB at optimum pH, they found the maximum rates of metal removal from acid mine drainage were 47.5 mg Cu/L/hr, 18.1 mg Zn/L/hr, and 25.7 mg Fe/L/hr.

Dvorak et al. (1992) conducted pilot-scale experiments to evaluate the potential of SRB for treating metal-contaminated water. Specific design requirements for the sulfate reduction reactors were:

- the exclusion of oxygen
- a source of sulfate (commonly present in contaminated water)
- a source of simple organic compounds to serve as a primary carbon source
- the presence of sulfate-reducing bacteria
- a way to physically retain metal sulfide precipitates.

The result of the preliminary study by Dvorak et al. (1992) indicated that pH <5 inhibited the activity of SRB and increased the solubility of metal sulfides.

Pilot-scale reactors were filled with loosely packed spent mushroom compost and installed to treat metal-contaminated water in an underground coal mine and at a smelting residue dump. Decomposing compost served as a source of organic carbon for SRB while the bulk materials physically retained precipitated solids. Dvorak et al. (1992) reported that these simple anaerobic reactors filled with bulk organic material lowered concentrations of Al, Cd, Fe, Mn, Ni, and Zn by over 95 percent while completely neutralizing the acidity of the contaminated water. The biosystem raised the pH of the liquid stream from 3.2 to 6.4. However, a major disadvantage of these systems is that the removed metals are retained in the bioreactors, and the systems give no means to recover the precipitated metals. Iron, nickel, and cadmium were retained within the reactors as insoluble sulfides after their reaction with biologically generated H_2S. Aluminum and manganese were retained as insoluble hydroxides or carbonates. Zinc was retained in both carbonate and sulfide phases. Dvorak et al. (1992) concluded that the precipitation of metal sulfide accounted for 36 to 76 percent of the total mass of metals retained within the sulfate reducing reactors.

Hammack and Edenborn (1993) suggested that the rate of metal removal and alkalinity generation might be enhanced by increasing the rate of bacterial sulfate reduction. They found that the rate of sulfate reduction and metal retention were increased by a factor of 10 following the addition of lactate, which is a preferred carbon source for many sulfate-reducing bacteria. Raising the reactor temperature into the

range of 25 to 35 °C to stimulate bacterial activity also increased the rate of sulfate reduction.

SRB also play a predominant role in the process of the biological treatment of acid mine drainage (AMD) using natural wetlands. This biological approach has been successfully applied to treat AMD at a deserted coal mine site in southern Illinois (Nawrot, Klimstra, and Sandusky 1990). More than 60 million gallons of acid water (pH 3.3, 263 ppm acidity) were discharged into the reedgrass wetland during a 10-month period. The wetland successfully neutralized the chromic acid input and removed iron, manganese, and other heavy metals from the aqueous stream as metal sulfide precipitates. The improvement in water quality was attributed to the bacterial sulfate reduction taking place at the anaerobic sediment zone of the wetland.

J.Z. Xie, D.K. Cha, and J.J. Kilbane (unpublished results, 1993) developed an SRB biosystem for the treatment of metal-contaminated wastewater. The system consisted of a biofilm bioreactor containing SRB to precipitate heavy metals from wastewater and a settling tank to collect metal precipitates as sludge. The study showed that SRB can effectively precipitate and remove metals from wastewater having pH values as low as 1.7. The system also tolerated high concentrations of toxic heavy metals (concentrations as high as 270 mg/L Cd have been tested) with metal removal efficiencies of 95 to 99 percent.

Haas and Chongchin (1993) conducted a laboratory study to develop a continuous precipitation-anaerobic biodegradation process. The biologically produced sulfides in the anaerobic reactor supernatant were recycled to the influent stream to precipitate the heavy metals in the incoming wastewater. They used copper as the metal of interest and simple organic compounds such as glucose and acetate as primary organic substrates; they found that the biologically produced sulfide in anaerobic reactor effluent was a better precipitant than inorganic sulfides. Their result showed the feasibility of using the sulfide precipitation-anaerobic digestion process for treating a waste containing high concentrations of both organics and metals.

Reduction of Hexavalent Chromium Under Sulfate-Reducing Condition

Smillie, Hunter, and Margaret (1980) reported that H_2S produced by sulfate-reducing bacteria in sediment can reduce Cr (VI) to less toxic Cr (III). They explained that this reduction process was the primary reason for the predominance of Cr (III) in sea water even though, from thermodynamic considerations, Cr (VI) should predominate. The resulting Cr (III) can easily be removed from solution by precipitation as oxides and hydroxides.

Allied Signal, Inc. (1992) developed a bioreactor that simulates the natural Cr (VI) reduction process occurring in the aquatic environment. The bioreactor consisted of a gravel layer for the activity of the sulfate-reducing bacteria, a sludge layer, and "treated" water above the sludge layer. The developers of the process claim that the effluents from the bench-scale tests typically contain less than 1 mg/L Cr (VI). However, Allied Signal's chromium reduction scheme requires a dilution of wastes to lower the incoming concentration of Cr (VI) to less than 200 ppm (because of the toxicity of Cr (VI) to microorganisms) and a continuous addition of carbon sources, sulfate, and nutrients.

Cited References

Allied Signal, Inc., "Bioremediation of hexavalent chromium wastewater," *The Hazardous Waste Consultant* (July/August 1992), p 130.

Dvorak, D.H., et al., "Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot-scale reactors," *Biotechnology and Bioengineering*, vol 40 (1992), p 609.

Hammack, R.W., and H.M. Edenborn, "The removal of nickel from mine waters using bacterial sulfate reduction," *Applied Microbiology and Biotechnology* (1993).

Maree, J.P., and W.F. Strydom, "Biological sulfate removal in an upflow packed bed reactor," *Water Research*, vol 19 (1985), p 1101.

Metzner, A.V., "Removing soluble metals from wastewater," *Water & Sewage Works* (April 1977), p 98.

Middleton, A.C., and A.W. Lawrence, "Kinetics of microbiol sulfate reduction," *Journal Water Pollution Control Federation*, vol 49 (1977), p 1659.

Murr L.E., A.E. Torma, And J.A. Brierley, *Metallurgical Application of Bacterial Leaching and Related Microbiological Phenomena* (Academic Press, New York, 1978), p 321.

Nawrot, J.R., W.B. Klimstra, and J.E. Sandusky, "Biochemical treatment of mine drainage through a reedgrass wetland," *Proceedings of the 1990 Mining and Reclamation Conference and Exhibition* (West Virginia University, Morgantown, WV, 1990).

Patterson, J.W., *Industrial Wastewater Treatment Technology*, 2d ed. (Butterworths, Boston, 1985).

Postgate, J.R., *The Sulphate-reducing Bacteria*, 2d ed. (Cambridge University Press, Cambridge, England, 1984).

Pugsley, E.B., et al., "Removal of heavy metals from mine drainage in Colorado by precipitation," *Water*, vol 107 (1970), p 75.

Rouse, J.V., "Removal of heavy metals from industrial effluents," *ASCE Journal of Environmental Engineering*, vol 102 (1976), p 929.

Simillie, R.H., K. Hunter, and L. Margaret, "Reduction of chromium(VI) by bacterially produced hydrogen sulfide in a marine environment," *Water Research*, vol 15 (1980), p 1354.

Stanier, R.Y., et al., *The Microbial World*, 5th ed. (Prentice-Hall, Englewood Cliffs, NJ, 1986).

Stumm, W., and J.J. Morgan, *Aquatic Chemistry*, 2d ed. (Wiley-Interscience, New York, 1981).

Thimann, K.V., *The Life of Bacteria*, 2d ed. (Macmillan, New York, 1963).

U.S. Environmental Protection Agency (USEPA), *Summary Report: Control and Treatment Technology for the Metal Finishing Industry: Sulfide Precipitation* (Industrial Environment Research Laboratory, Cincinnati, OH, April 1980).

Wakao, N., T. Takahashi, Y. Sakurai, and H. Shiota, "The treatment of acid mine water using sulfate-reducing bacteria," *Journal of Fermentation Technology*, vol 57 (1979), p 445.

Uncited References

Barth, E.F., M.B.Ettinger, B.V. Salotto, and G.N. McDermott, "Summary report on the effects of heavy metals on the biological treatment processes," *Journal of Water Pollution Control Federation*, vol 37 (1965), p 86.

Bryant, M.P., L.L. Campbell, C.A. Reddy, and M.R. Crabill, "Growth of *Desulfovibrio* in lactate or ethanol media low in association with H_2-utilizing methanogenic bacteria," *Applied Environmental Microbiology*, vol 33 (1977), p 1162.

Compeau, G.C., and R. Bartha, "Sulfate-reducing bacteria: principal methylators of mecury in anoxic estuarine sediment," *Applied and Environmental Microbiology*, vol 50 (1985), p 498.

Guyot, J.P., and A. Brauman, "Methane production from formate by syntrophic association of *Methanobacterium bryantii* and *Desulfovibrio vulgaris*," *Journal of Applied Environmental Microbiology*, vol 52 (1986), p 1436.

Hass, C.N., and P. Chongchin, "Biological sulfide prestripping for metal and COD removal," *Water Environmental Research*, vol 65 (1993), p 645.

Lawrence, A.W., and P.L. McCarty, "The role of sulfide in preventing heavy metal toxicity in anaerobic treatment," *Journal of Water Pollution Control Federation*, vol 37 (1965), p 392.

Lovley, D.R., and M.J. Klug, "Sulfate reducers can outcompete methanogens at freshwater sulfate concentration," *Applied Environmental Microbiology*, vol 45 (1983), p 187.

McInerney, M.J., and M.P. Bryant, "Anaerobic degradation of lactate by syntropic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of H_2 on acetate degradation," *AppliedEnvironmental Microbiology*, vol 41 (1981), p 346.

Nanniga, H.J., and J.C. Gottschal, "Properties of *Desulfovibrio carvbinolicus* sp. nov. and other sulfate-reducing bacteria isolated from an anaerobic purification plant," *Applied Environmental Microbiology*, vol 53 (1987), p 802.

Yoda, M., M. Kitagawa, and Y. Miyagi, "Long term competition between sulfate-reducing and methane-producing bacteria for acetate in anaerobic biofilm," *Water Research*, vol 21 (1987), p 1547.

5 Conclusion

Current metal treatment technology basically relies on physical and/or chemical processes, but biotechnology is a promising area for further development.

The literature was reviewed to determine the development and status of microbiological applications for the removal of metals from polluted water and solids. The major areas of microbiological metal removal studied were:

- microbial leaching of heavy metals from contaminated solids
- microbial sorption of heavy metals
- microbial precipitation of heavy metals under sulfate-reducing conditions.

This report can serve as a reference guide for future metal sludge-related research and development.

USACERL DISTRIBUTION

Chief of Engineers
ATTN: CEHEC-IM-LH (2)
ATTN: CEHEC-IM-LP (2)
ATTN: CECG
ATTN: CECC-P
ATTN: CECC-R
ATTN: CECW
ATTN: CECW-O
ATTN: CECW-P
ATTN: CECW-PR
ATTN: CEMP
ATTN: CEMP-E
ATTN: CEMP-C
ATTN: CEMP-M
ATTN: CEMP-R
ATTN: CERD-C
ATTN: CERD-ZA
ATTN: CERD-L
ATTN: CERD-M
ATTN: CERM
ATTN: DAEN-ZC
ATTN: DAIM-FDP

CECPW 22310-3862
ATTN: CECPW-E
ATTN: CECPW-FT
ATTN: CECPW-ZC

US Army Engr District
ATTN: Library (40)

US Army Engr Division
ATTN: Library (12)

US Army Europe
ATTN: AEAEN-EH 09014
ATTN: AEAEN-ODCS 09014

INSCOM
ATTN: IALOG-I 22060
ATTN: IAV-DPW 22186

USA TACOM 48397-5000
ATTN: AMSTA-XE

US Army Materiel Command (AMC)
Alexandria, VA 22333-0001
ATTN: AMCEN-F
Watervliet Arsenal 12189-4050
ATTN: SIDWV-ATD
Tobyhanna Army Depot 18466
ATTN: Environmental Office
Anniston Army Depot 36205
ATTN: Environmental Office
Kansas AAP 76357
ATTN: Environmental Office
Installations: (19)

FORSCOM
Forts Gillem & McPherson 30330
ATTN: FCEN
Installations: (23)

6th Infantry Division (Light)
ATTN: APVR-DE 99505
ATTN: APVR-WF-DE 99703

TRADOC
Fort Monroe 23651
ATTN: ATBO-G

Fort Belvoir 22060
ATTN: CETEC-IM-T
ATTN: CETEC-ES 22315-3803
ATTN: Water Resources Support Ctr
ATTN: Australian Liaison Office

USA Natick RD&E Center 01760
ATTN: STRNC-DT
ATTN: DRDNA-F

US Army Materials Tech Lab
ATTN: SLCMT-DPW 02172

USARPAC 96858
ATTN: DPW
ATTN: APEN-A

SHAPE 09705
ATTN: Infrastructure Branch LANDA

CEWES 39180
ATTN: Library

CECRL 03755
ATTN: Library

USA AMCOM
ATTN: Facilities Engr 21719
ATTN: AMSMC-EH 61299
ATTN: Facilities Engr (3) 85613

USAARMC 40121
ATTN: ATZIC-EHA

Military Traffic Mgmt Command
ATTN: MTEA-GB-EHP 07002
ATTN: MT-LOF 20315
ATTN: MTE-SU-FE 28461
ATTN: MTW-IE 94626

Military Dist of WASH
Fort McNair
ATTN: ANEN 20319

USA Engr Activity, Capital Area
ATTN: Library 22211

US Army ARDEC 07806
ATTN: SMCAR-ISE

Engr Societies Library
ATTN: Acquisitions 10017

National Guard Bureau 20310
ATTN: NGB-ARI

Naval Facilities Engr Command
ATTN: Facilities Engr Command (8)

8th US Army Korea
ATTN: DPW (8)
HQ USFK/EUSA/EPO
ATTN: FKEN-E 96205-0010

USA Japan (USARJ)
ATTN: APAJ-EN-ES 96343
ATTN: HONSHU 96343
ATTN: DPW-Okinawa 96376

416th Engineer Command 60623
ATTN: Gibson USAR Ctr

US Army HSC
Fort Sam Houston 78234
ATTN: HSLO-F
Fitzsimons Army Medical Ctr
ATTN: HSHG-DPW 80045

Tyndall AFB 32403
ATTN: HQAFCESA Program Ofc
ATTN: Engrg & Srvc Lab

USA TSARCOM 63120
ATTN: STSAS-F

American Public Works Assoc. 64104-1806

US Army CHPPM 21010
ATTN: MCHB-DE
ATTN: ENAEC-TS
ATTN: Technical Information Center

US Gov't Printing Office 20401
ATTN: Rec Sec/Deposit Sec (2)

Nat'l Institute of Standards & Tech
ATTN: Library 20899

Defense Tech Info Center 22304
ATTN: DTIC-FAB (2)

196
4/95